SEASONAL CHANGES IN THE GASES AND SUGAR OF THE BLOOD AND THE NITROGEN DISTRIBUTION IN THE BLOOD AND URINE OF THE ALLIGATOR*

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SEASONAL CHANGES IN THE GASES AND SUGAR OF THE BLOOD AND THE NITROGEN DISTRIBUTION IN THE BLOOD AND URINE OF THE ALLIGATOR

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The original purpose in investigating the blood of the alligator was to follow any changes occurring in the blood of a poikilothermic animal, when the temperature of the environment was varied. The carbon dioxide production and oxygen utilization in poikilothermic animals follow closely changes in surrounding temperatures, although there is evidence of regulatory control of metabolism within a range of temperature of from 15° to 20°C. (1). Complex changes occur in the blood of homoiothermic animals under conditions of high environmental temperatures, many of which are directly related to the mechanism of temperature control (2). Changes occurring in the blood following a change of environmental temperature ought to be more directly related to the environmental conditions in poikilothermic than in homoiothermic animals.

In the search for a suitable cold-blooded animal, the American alligator was selected because it offers the advantage of suitable size and the consequent possibility of supplying sufficiently large quantities of blood without serious disturbances due to hemorrhage.

Methods for securing the blood were worked out and preliminary analyses of the gases of the blood were made. It was found that the carbon dioxide contents were markedly lower in January and February, when the work was begun, than in the following March. In some cases there was a difference as great as twenty volumes per cent. In searching for a cause of these variations it occurred to us that they might be due to an annual rhythm, and it became necessary to temporarily discontinue the work as originally planned and to pursue the question of these seasonal variations.

The importance of seasonal changes is obvious in this connection, for it would be impossible to interpret the reaction of the animal to experimental conditions if it is at the same time responding to an unknown and uncontrolled cycle of changes, due not to its immediate environment, but to its individual or inherited history.

Extensive investigations of the nitrogen metabolism of birds have been carried out showing that uric acid is the end product of protein metabolism. Ammonia and lactic acid (3) are combined to form uric acid which also originates from amino acids (4), urea (5), ammonia (6) and from the purin bases (7).

Reptiles are generally classed with birds in their nitrogen metabolism since the excrement of the snake has been found to consist of ammonium urate (8). Among reptiles, however, there is a greater diversity in structure than among birds, and it was thought that investigation of the nitrogen end products in the alligator might add to our knowledge of the biochemistry of this class of animals.

Apparently the blood sugar of the alligator had never been determined previous to this investigation and it was felt that it should be included with the other factors studied.

Discussion of methods. *I. Biological. a. General care of animals:* We thought it advisable to maintain our animals under conditions as nearly uniform as was practical. A wooden zinc-linked tank was built, having dimensions of 5 feet × 2 feet × 2 feet. The cover was divided into three sections, the middle section being of glass, and the two end sections of heavy muslin, for ventilation. Small holes were bored through the upper portion of the tank to insure more complete ventilation. The temperature of the water in the tank was controlled by means of a hot water heating system, an electric stove being used as a source of heat. The temperature of the tank was maintained at approximately 26.5°C., a variation of 1 or 2 degrees occurring during sudden changes of weather. The water was changed once a week, and the tank cleaned. The water was heated to the proper temperature before being poured into the tank. The animals were fed twice a week during the spring and early summer, and weekly for the remainder of the period. Raw beef cut in strips was used exclusively. Each time the animals were used for experimental purposes, they were weighed and measured.

Seven alligators were kept in the tank during the fifteen months of the investigation, from January 1922 to March 1923, and at the end of the period they were to all appearances as healthy as at the beginning.

b. Method of drawing blood. Blood was drawn from the heart by means of a hypodermic needle with the precautions necessary for blood gas analysis according to the method described by Van Slyke (9). The heart beat is weak and difficult to detect by touch, and so the heart was located by means of a fluoroscopic examination. It was found to be well down in the middle of the body, between the ribs, and unprotected by the sternum. Later it was located in each animal by laying the animal on its back and examining it from the side, when a slight rise

and fall of the skin could be noted. This is essentially the same method as was used by Tiegel (10) in locating the heart of the snake. The horny scales of the epidermal skeleton are arranged in the ventral portion of the trunk in a very regular manner and it is possible by means of the transverse rows to locate the heart at the eleventh and twelfth row from the broad band just above the fore limbs. A needle two inches in length, gauge no. 20, was used. Coagulation was prevented by potassium oxalate.

Saturated oxalate solution was drawn through the needle just before using, and the needle dried by drawing air through it. To obtain the blood sample the needle is inserted through the skin, between the scales, for venous blood, about 0.5 cm. to the right of the midline of the body. When the purpose was to secure arterial blood, the needle was inserted 0.5 cm. to the left of the midline. In all cases, in searching for the heart the needle was directed parallel to the midline, being pushed gently through the tissue below the skin, very slowly, waiting for the detection of the heart beat. When the beating heart is felt at the end of the needle, a "diaphragmatic" expiration is awaited. The needle is then made to follow the heart downward. The needle is held in the final position, and the inspiration that follows pushes the heart upon the needle. By this technic the needle is inserted in the heart to approximately the same depth. Blood samples have been taken at intervals of two weeks throughout the year without the loss of a single animal.

c. Method of catheterization. Samples of urine were obtained by catheterizing the cloaca. The catheter was sterilized with 5 per cent carbolic acid, and inserted into the cloaca. The urine was collected in a small graduated cylinder, at times under oil. To prevent infection, the cloaca was washed out with 20 cc. of a saturated solution of boric acid, then washed with 20 cc. of sterilized distilled water.

In the alligator the rectum and ureters open into the cloaca. A sphincter muscle separates the rectum from the cloaca so that the cloaca acts as a bladder. The urine was collected many times, and was always found to be a limpid fluid and never contaminated with fecal matter.

II. Chemical methods. a. Blood gases. Estimation of the gases of the blood was carried out by means of the methods described by Van Slyke and Stadie (11). It was found necessary in making the oxygen determinations to add several more drops of ferricyanide than directed by Van Slyke, in order to completely liberate the oxygen of the blood; otherwise the method was followed exactly as described. The oxygen capacity of the blood was determined and the amount of hemoglobin calculated by multiplying by the factor 0.746. By a method for deter-

mining small amounts of iron, described by Brown (13), the hemoglobin was calculated from the iron and compared with that obtained by gas analysis, as a means of control. Instead of using alveolar air for the determination of the carbon dioxide capacity of the blood, samples of blood were exposed to a determined tension of 40 mm. of mercury of carbon dioxide. Ten minutes in a slowly rotating Squibb funnel were allowed for equilibration.

b. Blood sugar. The sugar of the blood was determined by the MacLean method (14), as modified by Hastings and Hopping (15).

c. Nitrogen of the blood. Total nitrogen of the blood was estimated by the Macro-Kjeldahl method (16). Urea was determined by the method described by Myers (17) and ammonia by the method also described by Myers (17), except that 3 cc. of blood were used instead of 2. In both cases the liberated ammonia was collected in N/50 H_2SO_4, and titrated against N/50 NaOH. Urea was determined also by Folin's method on the tungstic acid filtrate (18). Uric acid was determined by Benedict's new method (19), and in a few cases, by the Folin method. Creatine, creatinine and non-protein nitrogen were determined by Folin's method (18), the non-protein nitrogen also by a Macro-Kjeldahl method, titrating with N/50 alkali.

d. Urinary nitrogen. Total nitrogen of the urine was determined by the Macro-Kjeldahl method as described for blood. Urea was determined by the method of Van Slyke and Cullen (20). In carrying out the urea determination it was necessary to make the urine faintly acid to litmus before addition of urease. Ammonia was determined by the Steele modification of Folin's method (21). The ammonia content of the urine was so high that the equivalent of 0.2 cc. of urine was used for estimation of ammonia. Urease was used in tablets made by Squibb, also in powder form as made by the Arlington Chemical Company. Uric acid was determined by Benedict's latest method (21).

We wish to express here our appreciation of the help given us by Professor Benedict in carrying out this investigation.

e. Chlorides. The chlorides in the blood were determined by the method of Austin and Van Slyke (23). In the urine, the chlorides were determined by the method of Volhard-Harvey (24).

Discussion of results: *1. Health of animals.* The value of information gained by experiments carried out over a period of more than a year, upon animals kept under laboratory conditions and subjected to treatment that might be regarded as injurious, must depend largely upon evidence that the animals have not been rendered abnormal. The usual criteria by which such questions are decided are increase in weight and continued growth of the animal. Measurements of length were made over a period of six months and these showed an average increase

in length of 6 cm. for the period, or 1 cm. a month (table 1). According to measurements collected at the New York Zoölogical Garden, under the most favorable conditions, this animal was found to grow about a foot a year (25). Our figures are only 40 per cent of this number but, as mentioned above, the feeding of our animals was cut down considerably and was less than that as described at the Zoölogical Garden. Records of weight were kept and figure 1 shows that for each animal there was a gradual and steady increase. There is an exception to this statement in the earlier portion of the curve, in the case of animals 1 and 2. This was due to the difficulty experienced in finding suitable food. The flattening of the curve which is noted at the middle of the summer was due to the reduction in the amount of food at this time which was mentioned above. This reduction was necessary since the animals were becoming so large that they threatened to outgrow our facilities for handling them.

TABLE 1

Increase in length from June to December, 1922

NUMBER OF ANIMAL	INITIAL LENGTH	FINAL LENGTH	DIFFERENCE
	cm.	*cm.*	*cm.*
1	98.4	108.0	9.6
2	88.1	93.5	5.4
3	65.4	72.4	7.0
4	101.0	105.0	4.0
5	108.0	115.0	7.0
6	86.0	90.0	4.0
7	96.6	103.0	6.4
Mean			6.2

Since the animals gained in weight and increased in length it may be presumed that they were not seriously injured by the experimental conditions to which they were subjected.

2. General character of the blood. The blood drawn from the left side of the heart is bright red and that from the right side dark, purplish red in color. The general appearance of the blood is quite similar to that of the dog or man. The mean volume percentage of corpuscles is 14.5. Under the microscope the red corpuscle is a pale yellow, oval cell, with a large nucleus. The average sizes of the erythrocytes are given below.

Length	24.89–26.52μ
Width	12.24–16.32μ
Thickness at center	4.08μ

The corpuscles, thus, are larger than human corpuscles. The average number of red corpuscles in the seven animals studied is 850,000 per cu.mm. of blood. The extreme range in different individuals was 616,000 to 1,500,000. The observations from which these averages are obtained are given in table 2.

A leucocyte count was made on alligator 5 in the latter part of March, 1922, and was found to be 8,000 per cu.mm. of blood. The red count at that time was 760,000 per cu.mm. Toison's solution was used in making these blood counts since the leucocytes are stained by the methyl-violet in this solution. A year later a blood count was made on the same animal by Miss Morrison of the Vanderbilt Clinic. The count then was 760,000 for the red blood cells, but 446,000 for the

TABLE 2

Corpuscular volume and red cell count

NUMBER OF ANIMAL	CORPUSCULAR VOLUME		RED CELL COUNT	
	Date, 1922	Volume, per cent	Date, 1922	Number per cu. mm.
1	5-11	17.8	3-28	680,000
2	4-11	13.7	3-21	732,000
2	5-2	13.1	4-11	616,000
4	2-23	16.0		
5	2-23	15.8	3-23	730,000
5	4-6	14.0	3-21(1923)	760,000
6	3-2	11.4	3-13	881,000
6	5-9	11.4	3-30	800,000
6			4-18	745,000
7	4-18	16.1	3-13	1,480,000
7	5-9	15.5	4-4	1,130,000
7			4-25	1,050,000

leucocytes. Leucocyte counts were not made during the year, but a great increase in the number of leucocytes was observed when the red cells were counted. The ratio of leucocytes to red cells, on these determinations, is as 1 to 95, at the first observation, and about 1 to 2 on the second. In man, the values in normal blood are 5,000 to 9,000 per cu.mm. of blood, and about 5,500,000 erythrocytes, or a ratio of 1 to 600 or 1,100. Anything above 10,000 may be regarded as pathological, and in spleno-myelogenous leukemia the leucocyte count may rise to 500,000. It is an interesting fact that the animal that gave this high leucocyte count was in an apparently healthy condition. The study of this remarkable leucocytosis is being continued.

3. Blood gases. The oxygen contents and capacities of the blood and the carbon dioxide contents and capacities were determined throughout the year on six animals.

In following the oxygen content of the blood of an individual animal through a series of observations it is necessary to show that previous hemorrhage has presumably not modified the succeeding determinations.

Whipple (26) has shown that the hemoglobin is replaced in the dog following a hemorrhage of 50 per cent after about three weeks. No work upon the blood volume of the alligator has been reported but

TABLE 3

DATE	NUMBER OF ANIMAL	OXYGEN CAPACITY PER 100 CC. BLOOD		IRON PER 100 CC.
		By gas analysis	From iron	
		cc.	*cc.*	*mgm.*
17–10–22	1	13.2	14.0	34.2
12– 9–22	5	13.0	13.6	34.1
19– 9–22	5	11.2	11.8	29.7
13–10 22	6	12.4	12.2	30.5
3–10–22	7	10.1	10.4	26.1

TABLE 4

Seasonal changes in the gases and in the sugar of the blood

		1922						1923
		Jan.-Feb.	Mar.-April	May-June	July-Aug.	Sept.-Oct.	Nov.-Dec.	March
Volume per cent:								
Oxygen capacity	Mean	13.7	10.6	9.5	9.9	11.8	12.2	15.3
	St. dev.	2.5	2.0	2.5	1.3	1.4	0.6	2.7
Oxygen contents	Mean	11.0	5.5	1.8	3.5	4.3	5.7	10.9
	St. dev.	1.5	1.4	0.2	1.6	1.2	2.7	1.0
Carbon dioxide capacity	Mean	49.9	67.8	53.2	48.3	47.3	45.9	54.3
	St. dev.	5.9	5.3	12.3	3.7	7.1	3.6	7.6
Carbon dioxide contents	Mean	38.6	60.6	48.6	44.4	40.7	42.6	49.4
	St. dev.	5.5	4.9	12.6	5.5	3.8	2.4	16.1
Sugar in mgm. per 100 cc. of blood.	Mean	97.0	95.2	92.8	83.0	65.7	29.6	58.0
	St. dev.	27.7	23.6	21.5	26.4	11.8	10.8	10.2

Fry (27) has shown that of a lizard to be 3 per cent and of the frog to be 4.6 per cent of the body weight. If the blood volume of the alligator is assumed to be of this order, we have at no time drawn more than 12 per cent of the total blood volume, and as two weeks were allowed for recovery, it is felt that hemorrhage of itself was not a disturbing factor.

To check the accuracy of the method of gas analysis, the iron of the blood was estimated by the method of Brown (13). The oxygen capacity of the blood was then calculated from the iron and compared with that obtained by gas analysis. These figures are shown in table 3, and a satisfactory agreement is apparent.

4. Seasonal changes of blood gases. The mean values for the gases of the entire series, grouped in bi-monthly periods, are shown in table 4. A survey of the figures for oxygen capacity arranged in bi-monthly periods (table 4), shows that the oxygen capacity reaches a maximum in the early spring, and is lowest during the late spring and summer months. The value found in March of the second year is higher than that of the preceding summer, and is slightly higher (by about 5 volumes per cent) than that of the corresponding period of the preceding year. This increase is very probably due to the stimulation of the formation of hemoglobin due to repeated hemorrhage to which the animals had been subjected, and which is known to occur in birds and mammals (28).

The oxygen contents for the period of January and February have a value of 11.0 volumes per cent; in March and April of the same year of 5.5 per cent; and for May and June of 1.8 per cent, which is the lowest value observed. Thereafter there is a gradual increase until the value of 5.7 volumes per cent is attained in the November and December period. In the following spring the values are again high giving an average of 10.9 per cent for the series.

The oxygen unsaturation of the blood varies from 20 per cent during the January and February period through 58 per cent for March and April, to 81 per cent in May and June. From this it progressively decreased to 46 per cent for November and December. Determinations were not obtained during January and February of the second year but those made early in March gave a value of 29 per cent.

Any interpretation of these results must take into account the fact that the animals were kept at the approximate temperature of 26.5°C. No observations of the blood gases of the alligator in its natural habitat have been made but the differences we find during the year are such as we might expect to find if the animals were subjected to seasonal changes. In cold weather, the metabolism and consequent use of oxygen are at a low level and the oxygen unsaturation of venous blood would be slight. In summer months when metabolism is more rapid, we would expect the oxygen unsaturation also to be correspondingly greater, since the oxygen is consumed to a greater extent.

We must interpret these results as due to a periodicity acquired by the animal in its former habitat, or perhaps to some natural, internal rhythm of the animal, but certainly not to the immediate environment in which the animals lived during the period of observation.

The blood of different animals when exposed to carbon dioxide at a tension of 40 mm. of mercury shows a varying capacity for fixing this gas. The carbon dioxide capacity is distinctly higher in the early spring than during the remainder of the year. This is illustrated in table 4, from which it appears that the carbon dioxide of the March and April period is well above that of the other periods. The fact that the volume for the second March is lower than the corresponding earlier period is possibly due to a partial loss of the periodicity resulting from the prolonged period in which the animals were subjected to constant temperature conditions.

The carbon dioxide contents show a similar behavior. In the March and April period, carbon dioxide content is greater than in the winter or summer months. The lowest period seems to be in the fall and winter. In the second spring the value is higher than during the preceding winter and summer, but is not as high during the first spring. Animals 4,6 and 7 gave high values while the other three animals were low. The fact that some of the animals do not show an increase at this time of the year, while some do, is possibly due to a gradual loss of the periodicity due to constant conditions.

5. Gaseous nitrogen of the blood. The gaseous nitrogen of the blood was found to be higher than in any animal so far reported. In human blood, the average value for the nitrogen is 1.36 volumes per cent (29). In the horse, Regnard and Schlosing (30) found the nitrogen to be 2.00 volumes per cent. On ten determinations the mean value for the alligator was 0.033 with a standard deviation of 0.0055, on 1 cc. of blood, giving a value of 3.3 volumes per cent.

6. Chlorides. The chloride content of the blood of the alligator was found to be 525 mgm. per 100 cc. of blood. The value for dog's blood is about 550 to 570 mgm. per 100 cc.; for ox blood about 450 mgm., and for human blood 650 mgm. per 100 cc. The urine appeared to be wholly free from chlorides, not a trace being found at any time.

7. Relative efficiencies of erythrocytes of alligator and man. It was of interest to compare the relative efficiency of the erythrocytes of the alligator and man as oxygen carriers. This was done by comparing the actual size of the red corpuscles of the alligator with those of man, and at the same time, comparing each with respect to oxygen carrying power. From this it may be possible to form some idea of the physiological efficiencies of the cells of the two types of animal.

The volume of each erythrocyte may be calculated by dividing the corpuscular volume by the number of red cells. Thus in man, the corpuscular volume may be taken as 40 per cent (31) and the number of red cells per cubic millimeter as 5,500,000 (31). The volume occupied by one corpuscle would be 0.4 cu.mm. divided by 5,500,000 or 7.2 $\times$

10^{-8} (32). In the alligator a wide range in the number of red cells was found, but the average number per cu.mm. is 850,000, and the average corpuscular volume per cent is 14.5. The volume of the individual corpuscle, therefore, is 0.145 cu.mm. divided by 850,000 or 16×10^{-8}. The alligator corpuscles therefore compare in volume with the human corpuscles as $\frac{16 \times 10^{-8}}{7.2 \times 10^{-8}}$ or as 2.2: 1.

The oxygen capacity of human blood is about 19 cc. per 100 cc. of blood. The factor for converting oxygen capacity into hemoglobin is 0.746. The hemoglobin content of human blood is 14 to 15 grams per 100 grams of blood. The oxygen capacity of alligator blood may be taken as 12 cc. per 100, and the hemoglobin content would then be about 9 grams per 100 grams of blood. If a proportional amount of hemoglobin were carried by the two kinds of erythrocytes, the corpuscular volume would also be proportional. That is 9: 14.5 as X: 40. The corpuscular volume of the alligator under these circumstances would be about 25 volumes per cent. The corpuscular volume was found, however, to be 14.5 per cent and thus 25/14.5 represents the ratio between the hemoglobin carrying powers of the two kinds of corpuscles. The alligator corpuscles then either contain relatively more hemoglobin than human corpuscles, or in some way the hemoglobin is more efficient. From this we may conclude that one alligator corpuscle is equal in volume to 2.2 human corpuscles and in its efficiency as an oxygen carrier is 1.7 times that of the human corpuscle. So it would take about four human erythrocytes to equal one alligator corpuscle as an oxygen carrier.

8. Comparison of the gases of arterial and venous blood. In order to compare the gaseous content of arterial and venous blood, samples were drawn successively in the manner described above, with the minimum possible time interval between samples, since it was obviously impractical to draw the two samples simultaneously. This was done, perhaps unfortunately, at the season of the year when the animals showed the maximum variation between individuals. However the results are interesting and are included as the first observations of the kind upon the alligator.

The detailed results are shown in table 5. The carbon dioxide contents, given by alligator 4, which alone gave the average value for this season as shown in table 4, for venous blood was 38.4 volumes per cent, and 32.0 volumes per cent for arterial blood, a difference of 6.4. Similarly, the oxygen content of venous blood was 11 volumes per cent, and for arterial blood 15.6 volumes per cent, and the difference was 4.6.

Similar data are not available for many species though Buckmaster and Gardner (33) publish some figures for the cat, and Hastings (34),

among others, for the dog, while there are, of course, many observations upon man (35). For purposes of comparison some of these data are included in table 6. It must be borne in mind that values of this kind are never absolute but must vary with the nature of the metabolism of the several individuals at the time that the samples were taken.

Peters, Barr and Rule (36) quote respiratory quotients in human blood, of 1.62 to 0.34, and Harrop (37) reports values of 1.81 to 0.62.

9. Carbon dioxide absorption curve. Oxalated blood was exposed to atmospheres containing known percentages of carbon dioxide and air. Samples were removed and analyzed after equilibrating the blood with

TABLE 5

Arterial and venous blood of the alligator

NUMBER OF ANIMAL	DATE	VENOUS		ARTERIAL		DIFFERENCE		R.Q.
		CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2/O_2
3	2-16-23	33.0	10.5	25.8	13.7	7.2	3.2	2.2
4	2-16-23	38.4	11.0	32.0	15.6	6.4	4.6	1.4
6	2-13-23	66.5	10.0	59.1	14.6	6.5	4.6	1.43
7	2-15-23	48.3	4.1	41.1	10.1	7.2	6.0	1.2
Mean						6.8	4.6	1.47

TABLE 6

Gases in volumes per cent in arterial and venous blood of several animals

	VENOUS		ARTERIAL		DIFFERENCE		R.Q.
	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2/O_2
Human	48.0	12.0	40.0	20.0	8.0	8.0	1.00
Dog	40.5	14.3	38.3	19.3	2.2	6.0	0.37
Cat (etherized)	40.8	9.9	25.07	13.6	15.77	3.7	4.2
Alligator 4	38.4	11.0	32.0	15.6	6.4	4.6	1.4

the gaseous mixtures. The results are plotted in figure 2, by placing tensions (calculated from percentages) of carbon dioxide in millimeters of mercury, on the abscissae and volumes per cent of carbon dioxide absorbed by the blood, on the ordinates. Determinations of this kind were made on the blood of each of three animals. The three curves fall at different heights as would be expected from the differences in alkali reserve already noted. They are, however, approximately parallel and the middle curve does not depart greatly from that for human blood published by Christiansen, Douglas and Haldane (38), which is drawn from the mean of many observations and which is represented by plus marks (+) in the figure. From this one might conclude that the mechanism of carriage of carbon dioxide in the blood of the alligator is essentially the same as that of human and mammalian blood, in general.

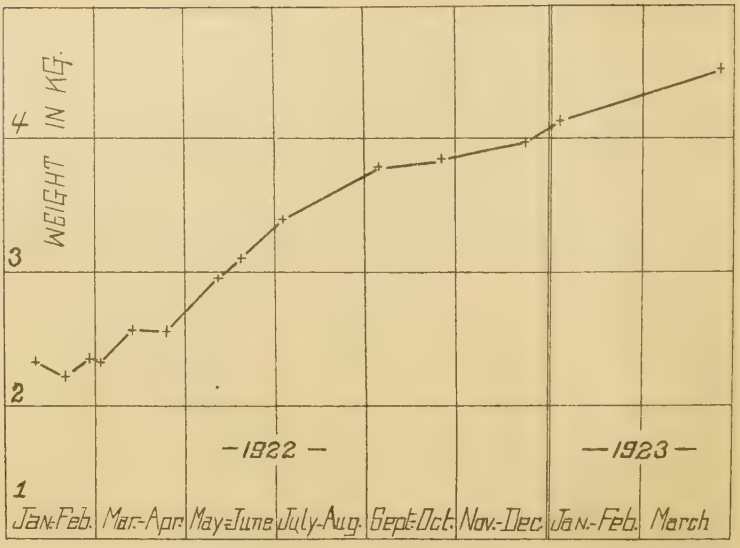

Fig. 1. Weight curve for alligator 1. The curves for the other individuals were essentially parallel to this. Only in two cases did the curves of the different individuals cross.

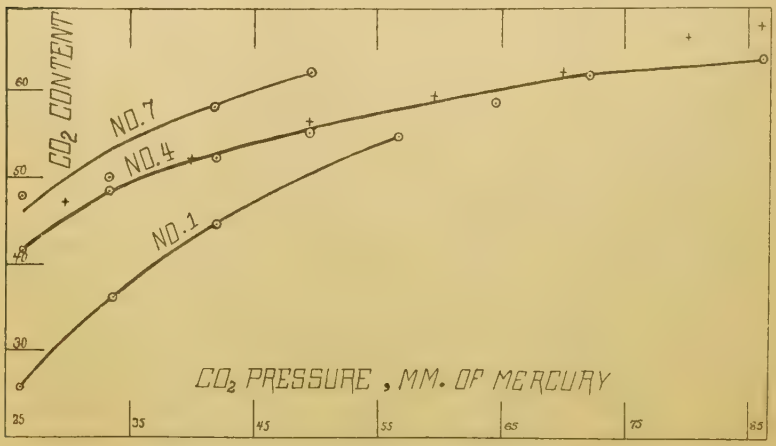

Fig. 2. Carbon dioxide dissociation curve for the blood of three alligators (circles) compared with that for human blood (crosses).

It might be further noted that the variations in alkali reserve among the individuals at this season are no greater than those found by Hastings (34) for different dogs.

10. Seasonal changes in the concentration of sugar in the blood. The concentration of the sugar in the blood of these animals was determined throughout the year. The mean values arranged in bi-monthly periods are shown in table 4. Animal 3, though subjected to the same general conditions, was used very little in the routine work of the study and served in the nature of a control on the disturbances due to the experimental procedure. The blood sugar in this animal was 97 mgm. per 100 cc. of blood in the January–February period, 92 mgm. in the July–August period, and 59 mgm. in the November–December period. In March of the second year the value was 92 mgm., about the same level as at the beginning of the experiment. The reaction of this animal was of the same nature as that of the other animals, but more marked, as will be seen upon comparison of the figures of this animal with those given in table 4.

A high sugar content is shown by all of the animals during the late winter and early spring. During the late spring a distinct drop in the sugar is noted, which continues during the summer and autumn, the lowest values being obtained for the November and December period. At this time, the mean for all the animals was only 37.5 mgm. per 100 cc. of blood.

Since the earlier determinations gave an average of about the same magnitude as is common in the mammals, i.e., 97 mgm., it is of interest to compare the low value with the "critical value" of 44 mgm. described by Banting, Best, Collip, Macleod and Noble (39). It should be noted that during this "low" period the alligators appeared in every way normal and so far as one could determine by appearance, were in the same physiological conditions as when the blood sugar was at its highest. The work reported by Riddle and Honeywell (40) for the pigeons indicates that the blood sugar of at least some forms can be reduced to a very low level indeed (25 to 30 mgm.) without evoking the disturbances which have been described for those of the mammals which have been studied. Alligator 1, on November 21, 1922, showed no sugar in its blood, though at the same time it appeared otherwise to be in normal condition, and on March 7, 1923, yielded a value of 113 mgm. This reminds one of the behavior of the dogfish where the sugar was at times low or absent. (See Scott (41) and Macleod (42).)

The annual cycle in the above discussed data is of much interest from the standpoint of seasonal changes in physiological phenomena, but any interpretation can only be conjectural at the present time.

The work of Riddle and Honeywell (43) shows a periodicity in the accumulation of sugar in the blood of the pigeon accompanying the periodicity of ovulation. The period of ovulation in the alligator occurs only in June, and only animals of mature age (five to ten years) carry out this function (44). All of our animals showed this seasonal rhythm, even the smallest which was hardly two years old, so in this case the periodicity in the blood sugar is evidently not related to ovulation.

The periodicity may be related to the storage of glycogen shown to occur during autumn and winter in the marmot by Claude Bernard (45) and in the frog by Moseik (46), since we found the sugar to be low at the season of the year that the glycogen in these animals is high.

In plants an accumulation of reserve substances occurs during the fall and winter, and a mobilization of these in a diffusible form occurs in the late winter and early spring (47), and in this respect may resemble a poikilothermic animal that passes through a winter period of lowered metabolism.

In all cases where the behavior of these alligators is related to hibernation, it is necessary to remember that they must have carried their periodicity over from their former habitat which was northern Florida, and hence our late winter period would correspond to their early spring.

In addition to any such conjectural considerations it is of course necessary to add the possible effects of the temperature changes in concentration of the blood sugar which may be presumed to be the results of such more immediate physiological equilibration.

11. Discussion of seasonal changes. There has grown up a very considerable literature on seasonal variations of physiological functions in various animals. In this literature it is developed that there are very few if any organs or physiological systems that are not affected by this factor in one or another of the animal groups. This is not the time for an extensive reivew of the literature though a brief review of some of the papers on variations in the respiratory system, the metabolism of the carbohydrates and the persistence of the seasonal variations after the animals have been removed from their natural and variable environment to the more or less perfectly controlled conditions of the laboratory may not be out of place. From the foregoing discussion it will be remembered that there was a periodicity in the concentration of the gases and the sugar of the blood of the alligator, which must be related to previous effect of changes in the seasons of the year, and not to the immediate environment of the animal.

The respiratory exchange in the lungs of the frog under laboratory conditions was found by Athanasiu (59) to varying according to the season of the year. He found that the respiratory quotient was greater in the winter months than in the summer.

During hibernation of the woodchuck, Rasmussen (48) found a gradual increase in the amount of carbon dioxide in venous blood from about 70 volumes per cent to as high as 100 volumes per cent. His results supported those of Dubois (49). When the animal wakes from its winter sleep the carbon dioxide falls very rapidly to its lower level.

In the frog there is a seasonal change in the storage of glycogen in the liver and other organs of the body. Mozeik (46) has shown that in summer there is scarcely a trace of glycogen in the liver, but in the late autumn the liver is extremely rich in glycogen. This observation was confirmed by Athanasiu (50). The period of high glycogen content in the frog corresponds to the period of low sugar content of the blood in the alligator.

TABLE 7

Nitrogen distribution in the urine

NUMBER OF ANIMAL	DATE	SP. GR.	MGM. PER 100 CC.				PER CENT TOTAL N		
			N	NH_3	Urea-N	Uric acid-N	NH_3	Urea	Uric acid
2	7–27	1.005	219	171	7.0	39.0	79.5	3.3	17.0
2	7 26	1.0062	249	198	3.3	28.0	80.0	1.3	11.0
1	8–4	1.0063	225	148	13.0	24.2	66.0	5.8	10.8
5	8–7	1.0048	268	208	16.0	18.4	77.0	6.0	7.0
			After sterile catheterization						
1	8–14	1.0049	208	168	0	41.3	81.0	0	19.8
1	11–8		207	140	20.5		67.0	10 0	
1	11–9	1.0053	202	154	35.0		77.0	17 0	

Claude Bernard (45) showed that in hibernating animals there is an increase in glycogen up to and including the period of winter sleep. The glycogen rapidly disappears when the animal awakes.

Physiological processes may be modified by a diurnal or annual cycle independently of the experimental conditions.

12. Distribution of the non-protein nitrogen in the blood and urine. The urine was collected by direct catheterization of the cloaca. The evidence that the urine so obtained was not contaminated by the feces and was in fact pure urine consists of the fact that it was always found to be a clear, limpid fluid, and that the specific gravity was found to be as consistent as would be expected. See table 7.

Analysis of the nitrogenous constituents of the urine always showed a high percentage of ammonia, in all cases more than 50 per cent of the total nitrogen appeared in this form, and occasionally more than 85 per cent.

Urea was usually present, in amounts varying up to 17 per cent of the total nitrogen, but was occasionally absent. Extra precautions for sterile catheterization tended to increase the proportion appearing in this form, but not in significant amounts.

Seven to 20 per cent of the total nitrogen of the urine appears as uric acid. The low percentage of uric acid is of interest in view of the common statement that the nitrogen metabolism of the reptiles is similar to that of the birds.

Wollaston in 1810 (51) showed that uric acid occurs in large amounts in the excrement of birds. Since this time, it has been shown by the investigation of a large number of workers that uric acid in birds arises from *a*, lactic acid and ammonia; *b*, from amino acids; *c*, from the purine bases, and *d*, from urea. Hoppe-Seyler showed that the excrement of certain lizards may consist almost wholly of uric acid (52), and Salkowski (53) mentioned the excrement of most snakes as a convenient source of

TABLE 8

Uric acid in blood and urine

NUMBER OF ANIMAL	BLOOD		URINE		
	Date	Mgm. uric acid per 100 cc.	Number of animal	Date	Mgm. uric acid per 100 cc.
1	4–20	1.67	1	7–25	36.8
1	5–11	2.25	1	7–26	37.6
4	5–4	2.56	1	8–2	27.8
5	4–27	1.83	1	8–3	23.6
5	5–18	2.60	1	8–4	24.2
6	5–9	1.79	1	8–14	41.3
7	5–16	2.24	1	10–24	22.8

obtaining uric acid in the pure form. The excrement of certain Pythons was shown to consist of ammonium urate (54). Snakes and lizards appear, therefore, to be similar to the birds in regard to the main end product of their protein metabolism. That this is not true of all forms of reptiles is shown, however, by the work of Karr and Lewis (55), who found large quantities of urea in the blood of the turtle. It is not true of the lower vertebrates, for nitrogen is largely eliminated by frogs (56) and fish (57) as urea. The facts just cited have given rise to the generalization that animals living in dry habitats, birds, snakes, lizards, excrete nitrogen as uric acid, while those living in water, fish, frogs and turtles, excrete it as urea instead. To this latter list we had hoped to add the alligator, but all we can say at the present time is, that it does not excrete large amounts of uric acid, and may possibly take an intermediate position between the lizards and the turtles.

The urine was neutral or faintly alkaline on drawing, but rapidly became strongly alkaline on standing, and a crystalline deposit of calcium carbonate and triple phosphates was thrown down. When collected under oil the urine showed a very high content of carbon dioxide, as much as 250 cc. per 100 cc. of urine. The change of reaction on standing is probably due in part to loss of CO_2.

We have found nothing in the literature on the nitrogen of the blood of the alligator, although Krukenberg (58) found creatine and hypoxanthin in the muscle extract of the alligator. We had hoped that analysis of the nitrogen constituents of the blood would throw light on the origin of the large quantities of ammonia in the urine. Contrary to our expectation, no urea was found in the blood. That this was not due to faulty technic is indicated by the fact that known amounts of urea added to the blood were recovered, and that the accepted values were found for beef blood. Less than 15 per cent of the non-protein nitrogen of the blood is in the form of ammonia. In other cases, where uric acid

TABLE 9

Nitrogen distribution in the blood

NUMBER OF ANIMAL	DATE	MGM. PER 100 CC.			PER CENT TOTAL N
		Non-protein N	NH_3	Urea	NH_3
2	1–4	15.0	2.1	0.7	14.0
1	1–5	15.0	2.1	0	14.0
7	1–9	19.6	2.1	0	10.7
4	1–10	18.0	2.1	0	11.7
6	1–11	16.0	2.1	0	13.0

alone was determined, the quantity of uric acid was found to average 2.13 mgm. per 100 cc. of blood, which represents only about 4 per cent of the non-protein nitrogen.

Neither creatine nor creatinine were found in either blood or urine.

The total nitrogen of the blood was found to be about 2000 mgm. per 100 cc. of blood.

The apparent absence of urea and the relatively small quantity of ammonia in the blood may indicate that there is some unknown precursor of the urea and ammonia which is found in the urine. The small amount of uric acid present in the blood would indicate that this precursor is probably not uric acid.

I wish to express my great appreciation of the continual assistance given me by Professor E. L. Scott of this Department, in carrying out this investigation.

SUMMARY

1. The erythrocytes of the alligator average 850,000 per cu.mm. of blood. They are about 14 μ in width and about 25 μ long, and are oval, nucleated cells.

2. The average corpuscular volume is about 14 to 15 per cent.

3. The oxygen capacity of the blood varies according to the season of the year between the values 9.5 and 13.7 volumes per cent. The oxygen content varies from 1.8 to 11 volumes per cent. The oxygen unsaturation of the venous blood is slight in late winter (20 per cent) and is very great in the summer (80 per cent).

4. The iron content of the blood is about 30 mgm. per 100 cc.

5. The carbon dioxide capacity and content of blood also show a seasonal variation, being about 20 volumes per cent higher in early spring than during the summer and winter.

6. The carbon dioxide absorption curve of alligator blood is essentially the same as that of human blood.

7. The sugar content of the blood shows a seasonal variation, being high in the spring and very low during late fall and winter.

8. Uric acid is present in only small quantities in the blood and urine of the alligator.

9. The main nitrogen constituent of the urine is ammonia. The precursor in the blood of the ammonia in the urine is not known.

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